

# PFAS Tiered Testing Strategy

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12/03/18

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*The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA*



## Tiered Toxicity & Toxicokinetic Testing

- Problem formulation:
  - Hundreds of environmentally-relevant PFAS without data
  - Traditional single-chemical toxicity testing not practical
  - Toxicity & toxicokinetic information needed to inform decision-making
- How do we generate informative toxicity and kinetic information quickly?
  - ORD, NTP, and partners have the capacity to generate toxicity and toxicokinetic data using high throughput screening (HTS) assays

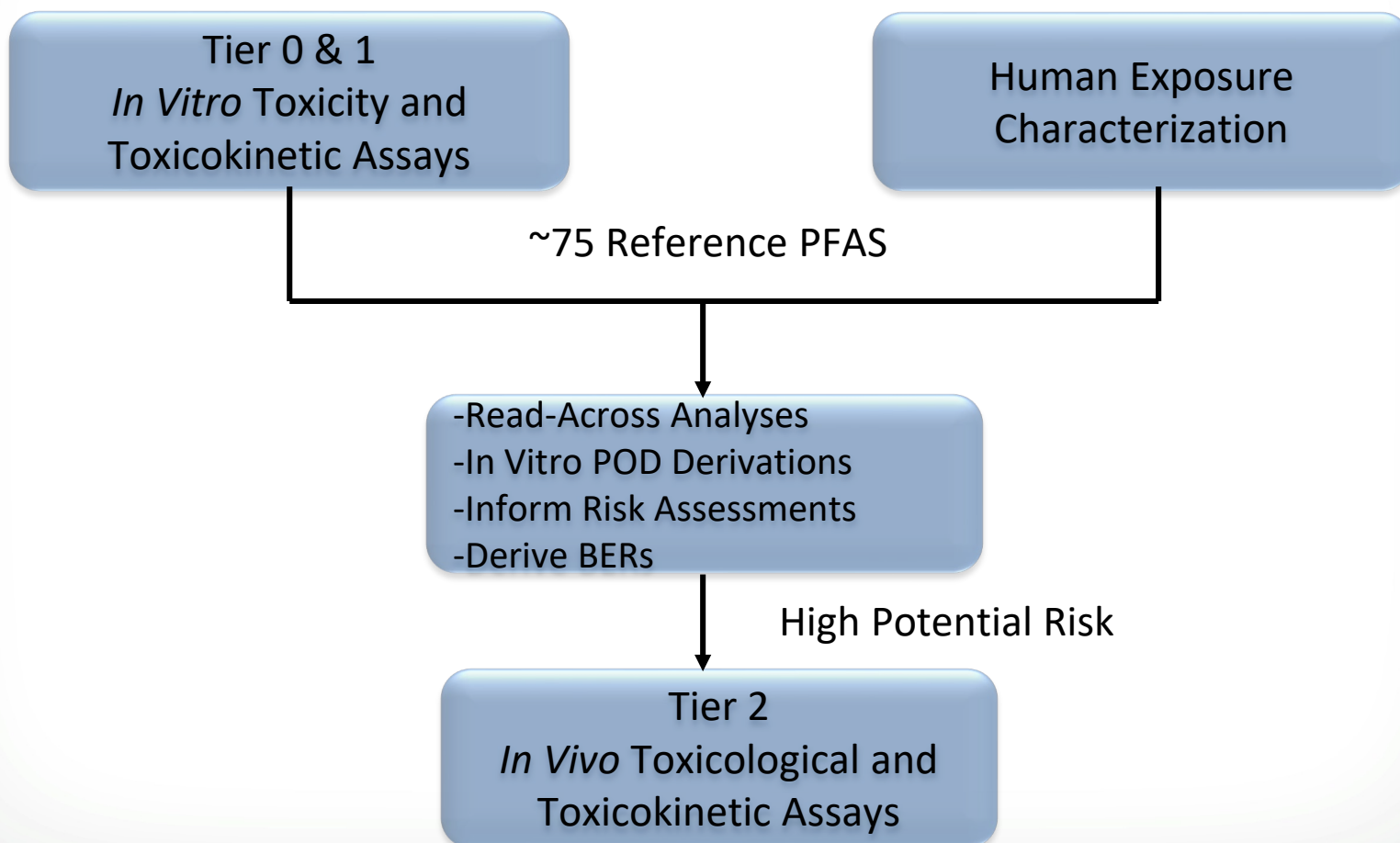


## Tiered Testing Overview

- Procure a library of ~300-400 PFAS (PFAS Screening Library) to support development of analytical methods, environmental monitoring, and toxicity testing
- Define a reference subset of PFAS
- Conduct tiered toxicity and toxicokinetic testing chemicals within the PFAS landscape
- Data will support multiple outputs, making use of the integrated dataset (in vivo, in vitro, etc.) to inform toxicity and kinetics across the PFAS landscape



# Tiered Toxicity and Toxicokinetic Testing Strategy



# PFAS Library and Chemical Selection

<https://comptox.epa.gov/dashboard>

**EPA DSSTox Database: > 758K chemicals**



- Chemical structures, downloadable files
- Predicted phys-chem properties
- External links & list overlaps

## PFAS Chemical Landscape:

DSSTox-registered >5000  
PFAS substances  
(>3 F, alkyl)

→ Spanning public lists  
of interest to EPA

~2000

→ Attempt to procure  
(chemicals w/ structures)

~430

→ PFAS Standards library  
(current library 430 PFAS)

← EPA's prioritized list for PFAS research  
(exposure, occurrence, health data)

~75

→ PFAS Reference subset  
(tiered toxicity & toxicokinetic testing)

## Per(poly)-fluorinated substances (PFAS)

- PFAS chemical names, acronyms, synonyms
- PFAS chemical structure categories

**PFOS**  
1763-23-1 | DTXSID3031864

© Searched by Approved Name: Found 1 result for 'PFOS'.

Wikipedia  
Perfluorooctanesulfonic acid (conjugate base perfluorooctanesulfonate) (PFOS)  
Read more

Intrinsic Properties  
Structural Identifiers  
Related Compounds  
Presence in Lists  
Record Information

Chemical Properties | Env. Fate/Transport | Toxicity Values (Beta) | ADME (Beta) | Exposure | Bioassays

Similar Molecules (Beta) | Synonyms | Literature | External Links | Comments

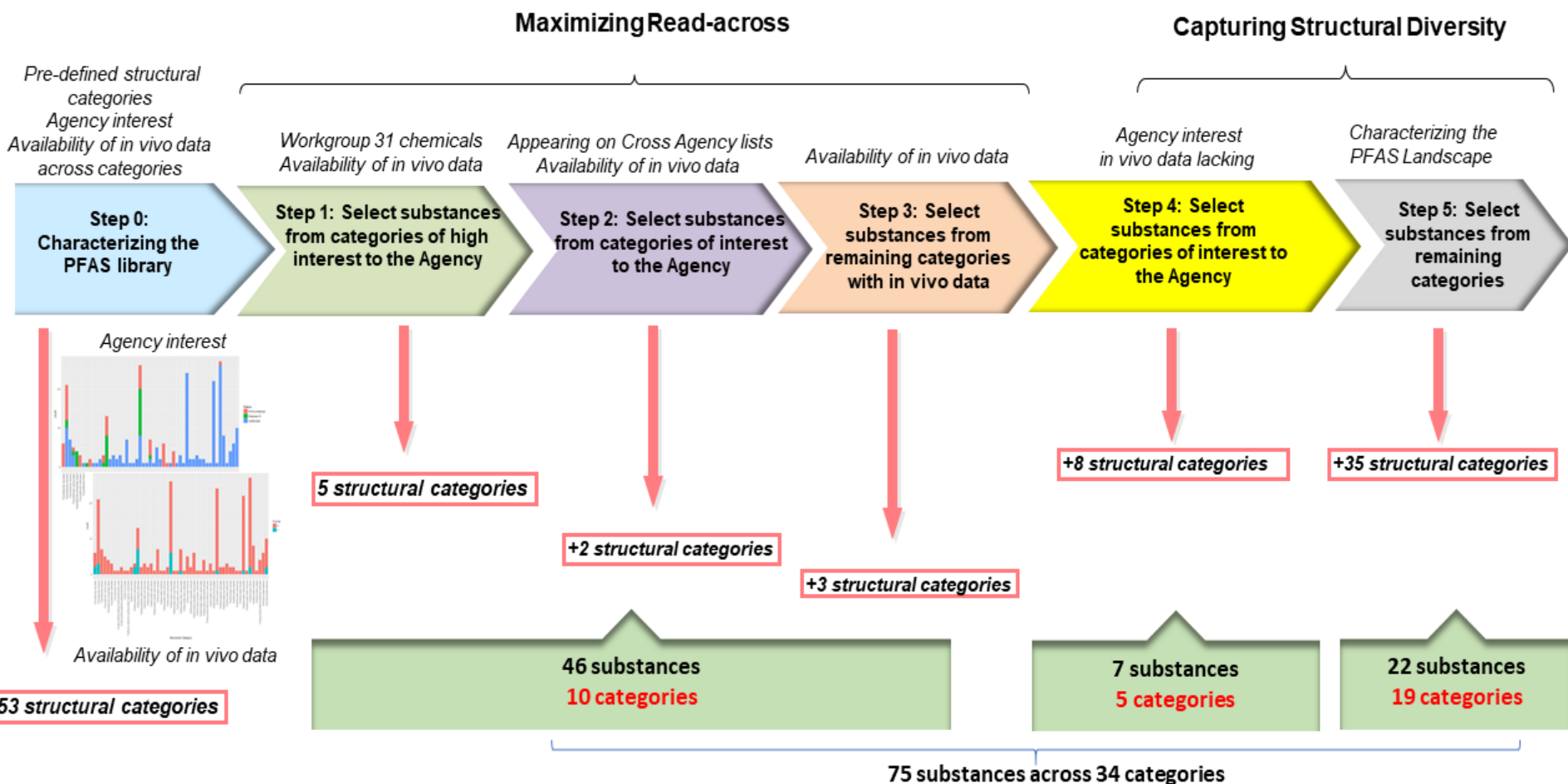
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 Potassium perfluorobutanesulfonate 29420-49-3	 Ammonium perfluorooctanoate 3825-26-1	 Potassium perfluorohexanesulfonate 3871-99-6	 10:2 Fluorotelomer acrylate 17741-60-5
 6:2 Fluorotelomer acrylate 17527-29-6	 Perfluorooctanesulfonamide 754-91-6	 Perfluorodecanesulfonic acid 335-77-3	 Pentafluoroiodoethane 354-64-3



# Selecting the Subset of 75 PFAS

Workflow for selection of structural categories containing the reference subset of 75 PFAS for tiered toxicity and toxicokinetic testing





## Tiered Testing: *In Vitro* Toxicity Assays

Endpoint of Interest	NTP	EPA	
		NCCT	NHEERL
Genome-Wide Screening (Tier 0)		High Throughput Transcriptomics (Httr) in 2 cell types and Phenotypic Profiling	
Hepatotoxicity	2D HepaRG cytotoxicity; 3D HepaRG (spheroid) transcriptomics	Attagene cis- and trans-Factorial assay (HepG2)	
Developmental Toxicity			Zebrafish embryo assay (ZEA)
Immunotoxicity	Bioseek Immune panel		
Mitochondrial Toxicity	2D HepaRG mitochondrial dye and Seahorse assay		
Developmental Neurotoxicity			Neurodevelopmental microelectrode assay (MEA)



## Tiered Testing: *In Vitro* Toxicokinetic Assays

Endpoint of Interest	NTP	EPA	
		NCCT	NERL
Hepatic Clearance	Human primary hepatocyte clearance		
Plasma Protein Binding			Ultracentrifugation assay
Renal Transport	Renal proximal tubule permeability assay		
Enterohepatic Recirculation		Piloting hepatic transporter assay	
<i>In Vitro</i> Disposition	Cell vs. nominal concentration (Tox21 joint project)	Cell vs. nominal concentration (Tox21 joint project)	



## PFAS Lists Added to CompTox Chemicals Dashboard – live Aug 2018

### PFAS MASTER LIST

- 7 component lists (EPA, EU, International)
- 5070 total unique substances
  - 5057 with CAS
  - 3992 with chemical structures
- EPA research & test landscape
  - 430 in EPA ToxCast Inventory (soluble in DMSO)
  - 43 in EPA ToxCast Inventory (insoluble in DMSO)
  - 126 different form (salt, anion) or unable to procure

PFASMASTER LISTS	PFASOECD	PFASKEMI	PFASTRIER	EPAPFASRL	EPAPFASINV	EPAPFASINSOL	EPAPFAS75S1	
PFASOECD	4730							OECD PFAS List
PFASKEMI	2206	2396						KEMI (Swedish Chem Agency) PFAS List
PFASTRIER	493	578	592					Community PFAS List (2015)
EPAPFASRL	132	116	71	199				EPA PFAS Research List
EPAPFASINV	309	324	226	61	430			EPA PFAS Inventory (DMSO Soluble)
EPAPFASINSOL	43	42	24	12	0	43		EPA PFAS Inventory (DMSO Insoluble)
EPAPFAS75S1	51	47	38	25	74	0	74	EPA PFAS 75 Test Sample (Set 1)



## Summary & Progress Overview

- Procured and established PFAS Chemical Screening Library – 430 compounds – Complete ✓
- Established process to optimize future *read-across* efforts and selected subset of 75 PFAS ✓
- Developed commentary on selection process and tiered testing for submission to *Environmental Health Perspectives*- Accepted ✓
- Plated and shipped PFAS to all labs conducting testing ✓
- Multiple PFAS lists processed through data curation and added to Comptox Chemistry Dashboard (5070 unique substances) ✓
- Highthroughput testing underway in most labs and anticipate raw data Winter 2018/19
- Selecting an additional 75 PFAS with the addition of HTTr and phenotypic profiling for the complete set (total of 150)- in process



## Acknowledgments

- NIEHS/NTP
    - John Bucher
    - Chad Blystone
    - Mike DeVito
    - Sue Fenton
    - Dori Germolec
    - Eric Tokar
  - USEPA
    - Rusty Thomas
    - Reeder Sams
    - Tina Bahadori
    - Jeff Frithsen
    - Annette Guiseppi-Elie
    - Amy Benson
    - Susan Burden
    - Lynn Flowers
    - Linda Gaines
    - Andy Gillespie
  - USEPA (continued)
    - Maureen Gwinn
    - Ron Hines
    - Jason Lambert
    - Pam Noyes
    - Grace Patlewicz
    - Stephanie Padilla
    - Ann Richard
    - Bruce Rodan
    - Tim Shafer
    - Mark Strynar
    - Tamara Tal
    - Barbara Wetmore
- and many others....*